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Original Article

Antimicrobial Resistance Pattern of *Stenotrophomonas maltophilia* Isolates from a Tertiary Care Setting in Rawalpindi, Pakistan

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Introduction: *Stenotrophomonas maltophilia* is intrinsically resistant to many antimicrobials. Like *Pseudomonas* spp., this bacterium has a remarkable ability to cause infections, particularly in the respiratory and urinary tracts. This study aims to determine the antimicrobial resistance pattern of *S. maltophilia* isolates collected from a tertiary care setting and frequency of multi, extensively and pandrug-resistant *S. maltophilia.* **Methods:** A cross-sectional study was carried out in Department of Microbiology, Fauji Foundation Hospital, Rawalpindi, Pakistan from January to June 2016. The isolates were identified as *S. maltophilia* using standard microbiological techniques, and the antimicrobial resistance was carried out using E-strip test against various antimicrobials. The data was analyzed and interpreted regarding frequencies and percentages. **Results:** Out of 90 isolates confirmed as *S. maltophilia*, pus (33.33%) and urine (24.44%) were the most common specimens from which this bacterium was isolated. Antimicrobial resistance pattern showed a high percentage of resistance to many antimicrobials with exception to aztreonam, minocycline, polymyxin B and colistin. **Conclusion:** Various *S. maltophilia* isolates from our set-up were resistant to antimicrobial agents used in the study. It is predicted that the infections caused by this bacterium shall be difficult to treat in the near future due to resistance to these antimicrobial agents. Though at this point no pandrug-resistant *S. maltophilia* is reported, the resistance pattern suggests that pandrug-resistant strains may appear shortly and when the time comes only newer antimicrobials can provide the answer. *J Med Microbiol Infec Dis, 2016, 4 (3-4): 83-87.*

Keywords: Antimicrobials, Pandrug-Resistance, *Stenotrophomonas maltophilia.*

INTRODUCTION

Stenotrophomonas maltophilia was known as *Pseudomonas maltophilia* in the past, but later found its place both in microbiological taxonomy and hospital infections [1]. This gram-negative aerobic bacillus can survive in water reservoirs. Contaminated endoscopes, dialysis units, and hospital water supplies are among the common reservoirs of this pathogen. The ability to form biofilms has been attributed to its existence on inanimate objects [1]. *Stenotrophomonas maltophilia* infects debilitated patients in hospitals particularly those with neutropenia or cystic fibrosis. Chronic respiratory illness, invasive procedures, immunosuppressive drugs, and prolonged hospitalization are other risk factors [1]. Infections by this pathogen include respiratory and urinary tract infections, cholecystitis, bloodstream, soft tissue and intraocular infections [2]. Like *Pseudomonas aeruginosa* it also targets burn patients, causing infections to the already weakened host [3].

Stenotrophomonas maltophilia has gained importance in recent years as it is intrinsically resistant to many antimicrobial agents including anti-pseudomonal penicillins, third generation cephalosporins and aminoglycosides [4]. This resistance has been attributed to various mechanisms including beta-lactamases, drug modifying enzymes, efflux pumps and mutations at drug target site [1]. This resistance has made a narrow choice for selection of antimicrobial chemotherapy. *Stenotrophomonas maltophilia* has also been known to coexist with *P. aeruginosa*, *Acinetobacter baumannii*, and Methicillin-resistant *Staphylococcus aureus* causing polymicrobial infections which may be difficult to treat [1]. Co-trimoxazole has been efficiently used in saving lives of the patients infected by this pathogen. However, the side effects and allergies to this antimicrobial have highlighted the need for new treatment options [4]. Multidrug-resistance (MDR) is defined as resistance to three antimicrobial agents, extensively drug-resistance (XDR) as resistance to all antimicrobials except one or two antimicrobials and pandrug-resistance (PDR) as resistance to all antimicrobials tested [5]. The objective of this study is to determine the antimicrobial resistance pattern of *S. maltophilia* and the frequency of multi, extensively and pandrug-resistant *S. maltophilia* isolates obtained in Fauji Foundation Hospital, Rawalpindi Pakistan in first half of 2016.

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MATERIAL AND METHODS

Isolation and identification of *S. maltophilia.* A cross-sectional study was done from January to June 2016 at Microbiology Laboratory, Department of Pathology, Fauji Foundation Hospital, a tertiary care hospital in Rawalpindi, Pakistan. Ethical approval was obtained from ethics and medical research review committee of the Foundation University Medical College, Pakistan to which the hospital is affiliated. Isolates were identified as *S. maltophilia* from some clinical specimens including pus, blood, sputum, urine, bronchial wash, tissue fluid, tracheal tube, high vaginal swab and intravascular cannula. All duplicate samples were excluded from the study. *Stenotrophomonas maltophilia* was identified by non-fermenting growth and by colony morphology on MacConkey agar followed by Gram staining. Biochemical tests were performed, and finally, the species were determined by Analytical profile index-20 Non-Enterobacteriaceae (API NE, BioMerieux, UK).

Antimicrobial susceptibility tests. Antimicrobial susceptibility was performed by E-strip method as guided by Clinical and Laboratory Standards Institute (CLSI) [6]. Polymyxin B, colistin, ciprofloxacin, aztreonam, and minocycline were used. Cefoperazone/sulbactam was tested only for isolates received from burn ward and intensive care unit. The incubation was done at 35°C ± 2 for 20-24 h. Minimum inhibitory concentration (MIC) was determined and interpreted according to the guidelines of CLSI [6].

Statistical analysis. Convenient sampling was done and all non-duplicate isolates during the study period were included in the survey. The data was analyzed by SPSS (version 21) software.

RESULTS

Isolation and identification of *S. maltophilia.* Ninety isolates were identified as *S. maltophilia* from various clinical specimens received from different hospital departments. The burn ward 23.33% (n=21) was the single most common site from where these specimens containing *S. maltophilia* isolates were collected. This was followed by medical wards 21.11% (19), chest ward/OPD 14.44% (13), pediatric ward 14.44% (13), ICU 11.11% (10) and nephrology ward 5.55% (5). The least number of isolates were obtained from surgical ward 3.33% (3), gynecology OPD 3.33% (3), cardiac care unit 2.22% (2) and orthopedics ward 1.11% (1). Isolation rates of *S. maltophilia* from other hospital departments are shown in Table 1.

The pus was the most common sample for isolation of *S. maltophilia*. Among all samples, pus contributed to 33.33% (n=30) of isolates, followed by urine 24.44% (n=22), sputum 23.33% (n=21), blood 7.77% (n=7) and tissue fluid 4.44% (n=4). Also, 2.22% of isolates (n=2) were obtained from the bronchial wash, tracheal tube and intravenous cannula. The distribution of *S. maltophilia* isolates in clinical specimens is given in Table 2.

Antimicrobial susceptibility tests. In our study, various antimicrobials were tested, and resistance was recorded (Table 3). The results revealed that resistance to all antimicrobials was more in burn ward than in ICU or other hospital wards. Most of the isolates were sensitive to colistin (0% resistance in ICU and burn ward and 5% in other hospital departments) and polymyxin B (9.5% resistance in the burn ward and 0% in ICU and other hospital departments). All the isolates from burn ward and ICU were resistant to cefoperazone/sulbactam. The resistance to ciprofloxacin was also 100% in burn ward, but 70% in ICU and 64% in other hospital departments. The resistance to minocycline was 71.4%, 40% and 22.03% in the burn ward, ICU, and other hospital departments. For aztreonam, less resistance was encountered in burn ward (only 14%) while the resistance reached 80% in ICU and other hospital departments.

Table 1. The rate of *S. maltophilia* isolation from various hospital departments

Hospital Department	No. of obtained isolates (%)
Burn ward	21 (23.33%)
Medical wards	19 (21.11%)
Pediatrics OPD	13 (14.44%)
Chest OPD/Ward	13 (14.44%)
Intensive care unit	10 (11.11%)
Nephrology ward	5 (5.55%)
Surgical ward	3 (3.33%)
Gynecology OPD	3 (3.33%)
Cardiac care unit	2 ((2.22%)
Orthopedic surgery ward	1 (1.11%)
Total sample	90 (100%)

Table 2. Distribution of *S. maltophilia* isolates in clinical specimens from different departments (n=90)

Specimen	Burn Ward No. (%)	ICU No. (%)	Other hospital departments* No. (%)	Total No. (%)
Pus	20 (22.22%)	4 (4.44%)	6 (6.66%)	30 (33.33%)
Urine	-	1 (1.11%)	21 (23.33%)	22 (24.44%)
Sputum	-	-	20 (22.22%)	20 (22.22%)
Blood	-	3 (3.33%)	4 (4.44%)	7 (7.77%)
Tissue fluid	1 (1.11%)	-	3 (3.33%)	4 (4.44%)
Bronchial wash fluid	-	-	2 (2.22%)	2 (2.22%)
Tracheal Tube	-	2 (2.22%)	-	2 (2.22%)
Cannula	-	-	2 (2.22%)	2 (2.22%)
High vaginal swab	-	-	1 (1.11%)	1 (1.11%)

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Table 3. Antimicrobial resistance pattern of *S. maltophilia* obtained from different departments

Antimicrobial agent	Resistance in Burn ward n=21 n (%)	Resistance in ICU n=10 n (%)	Resistance in other Hospital Departments* n=59 n (%)
Ciprofloxacin	21 (100%)	7 (70%)	38 (64.406%)
Cefoperazone/sulbactam	21 (100%)	10 (100%)	-
Minocycline	15 (71.428%)	4 (40%)	13 (22.033%)
Azetronam	3 (14.28%)	8 (80%)	48 (81.35%)
Polymyxin B	2 (9.523%)	0 (0%)	0 (0%)
Colistin	0 (0%)	0 (0%)	3 (5.084%)

*Other hospital departments include medical wards, pediatric OPD, chest OPD, chest ward, nephrology ward, surgical ward, gynecology OPD, cardiac care unit and orthopedic surgery ward.

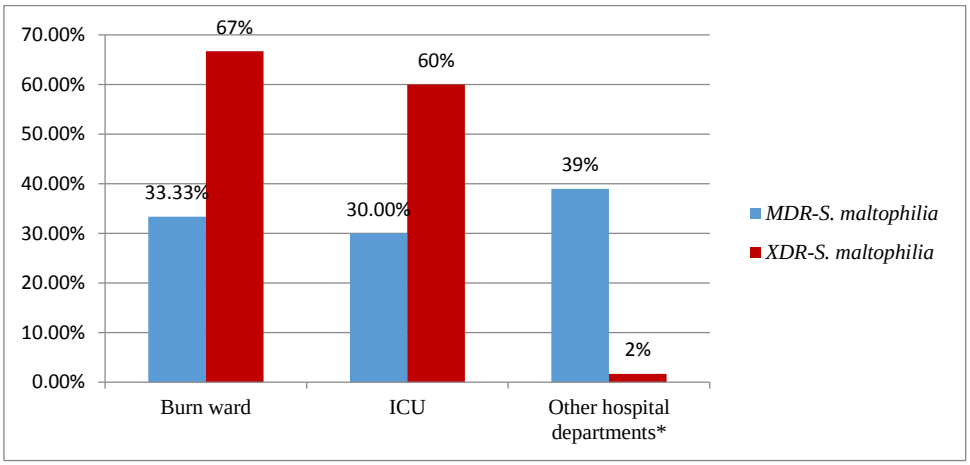


Fig. 1. The frequency of MDR and XDR- *S. maltophilia* among isolates obtained from different departments of the hospital

*Other hospital departments include medical wards, pediatric OPD, chest OPD, chest ward, nephrology ward, surgical ward, gynecology OPD, cardiac care unit and orthopedic surgery ward.

Antimicrobial susceptibility tests. In our study, various antimicrobials were tested, and resistance was recorded (Table 3). The Results revealed that resistance to all antimicrobials was more in burn ward than in ICU or other hospital wards. Most of the isolates were sensitive to colistin (0% resistance in ICU and burn ward and 5% in other hospital departments) and polymyxin B (9.5% resistance in the burn ward and 0% in ICU and other hospital departments). All the isolates from burn ward and ICU were resistant to cefoperazone/sulbactam. The resistance to ciprofloxacin was also 100% in burn ward but 70% in ICU and 64% in other hospital departments. The resistance to minocycline was 71.4%, 40% and 22.03% in the burn ward, ICU, and other hospital departments. For aztreonam, less resistance was encountered in burn ward (only 14%) while the resistance reached 80% in ICU and other hospital departments.

The frequency of MDR and XDR- *S. maltophilia* isolates in burn ward were 33.3% and 67%, respectively. The same trend was observed in ICU with 30% of isolates as MDR and 60% as XDR. However, in other hospital departments, XDR constituted only 2% of isolates with MDR reaching 39%. There was no PDR- *S. maltophilia* in the isolates of our study. Figure 1 shows the percentage of MDR and XDR *S. maltophilia* obtained from different hospital departments.

DISCUSSION

Stenotrophomonas maltophilia is now grouped along with *P. aeruginosa* and *A. baumannii* as the third most important cause of healthcare-associated infections [7]. In our study, 90 isolates were identified as *S. maltophilia* in a short period of six months mainly from pus samples, urine, and respiratory tract. In a survey carried out in Hungary, 68% of the isolates were obtained from respiratory tract specimens namely, tracheal aspirate, bronchoalveolar lavage, and sputum, in the order of frequency with which they were isolated [7]. A study conducted in Greece has reported that 54.4% of all infections of *S. maltophilia* were from lower respiratory tract [2]. Farrell *et al.*, concluded that among all infections by this pathogen 37% occur in respiratory tract being outnumbered only by blood stream infection, *i.e.*, 51% as opposed to our findings where only 7.7% of isolates were identified from blood samples [8]. It is reported that secondary blood stream infections can occur from burn and respiratory tract infections [9]. In our study, 24.4% isolates were cultured from urine samples. However, in another research urine samples contributed only 4.4% of all clinical specimens [2].

Juhász *et al.*, reported that 70% of the cultures positive for *S. maltophilia* were obtained from ICU whereas in our setting only 9% of these specimens were sent from ICU [7].

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Samonis *et al.* revealed that 20.6% of patients infected with *S. maltophilia* were hospitalized to ICU after the infection had occurred [2]. In our study, 21.11% percent of specimens containing this particular bacterium were received from medical ward as opposed to 52.9% reported by Samonis and colleagues [2].

The *in vitro* efficacy of various antimicrobials showed that the antimicrobial resistance of this pathogen was more in burn ward than the ICU and other hospital departments. A study from Hungary revealed 54% of resistance to ciprofloxacin in strains isolated from infected patients and 76% in strains isolated from colonizers [7]. A similar study in Pakistan reported 9.6% resistance to ciprofloxacin which is much lower than the rate obtained in our research [10]. Minocycline though effective in other hospital-acquired infections like methicillin-resistant *S. aureus* and multidrug-resistant *A. baumannii* [11-12] failed to show reasonable *in vitro* efficacy against *S. maltophilia* in our study. Most resistance to this antimicrobial was seen in the isolates from burn ward and ICU, the resistance to minocycline was 71.4% in the burn ward, 40% in ICU and 22.03% in other hospital departments. In a study from Korea, no strain of *S. maltophilia* was found resistant to minocycline [13]. This remarkable difference endorses the need for judicious use of these antimicrobials in our set-up to avoid positive selection of resistant strains. Here, we report low resistance rate to aztreonam in the burn ward, but resistance elsewhere in the hospital was high. From all the antimicrobials tested in this study, the least resistance was noted against polymyxin group of antibiotics. The previous report from Pakistan declared no resistance to colistin, but in our set-up, few isolates were found resistant both to colistin and polymyxinB. In the study by Juhász and colleagues resistance to colistin varied from 91% in infected patients to 77% in colonizers which is devastating [4]. However, there are reports showing as low as 9.8% resistance to colistin [2]. Likewise, widespread resistance to polymyxin B has also been noted. Farrell *et al.* reported 14.9% resistance to polymyxin B from Latin America to 57.7% in Asian-Pacific region [8]. Cefoperazone/sulbactam was applied only to isolates from burn ward and intensive care unit. More resistance was encountered in the burn ward. Wang *et al.,* have found 22% resistance of cefoperazone/sulbactam which is much lower than resistance noted in the burn ward in our study [14].

Based on our results and the report by Farell *et al.,* it is predicted that resistance of *S. maltophilia* to colistin and polymyxin might arise due to the selection of the resistant strains [8].

The frequency of MDR, XDR, and PDR- *S. maltophilia* is not reported in the medical literature, but their presence is widely known [1]. At this point, MDR- *S. maltophilia is* frequently isolated from all the hospital departments, and this is the point where medical researchers should quest for newer antimicrobials, not at the point of XDR or PDR- *S. maltophilia* prevalence.

Stenotrophomonas maltophilia isolates from our set-up were resistant to many antimicrobial agents used in the study. It is predicted that the infections caused by this bacterium will be difficult to treat in future due to resistance to these antimicrobial agents. Though presently no report of pandrug-resistant *S. maltophilia* is available, the resistance pattern suggests that pandrug-resistant strains may arise in the near future and when the time comes only newer antimicrobials can provide the answer.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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